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ENCAPSULATION IN FOOD PROCESSING AND FERMENTATION

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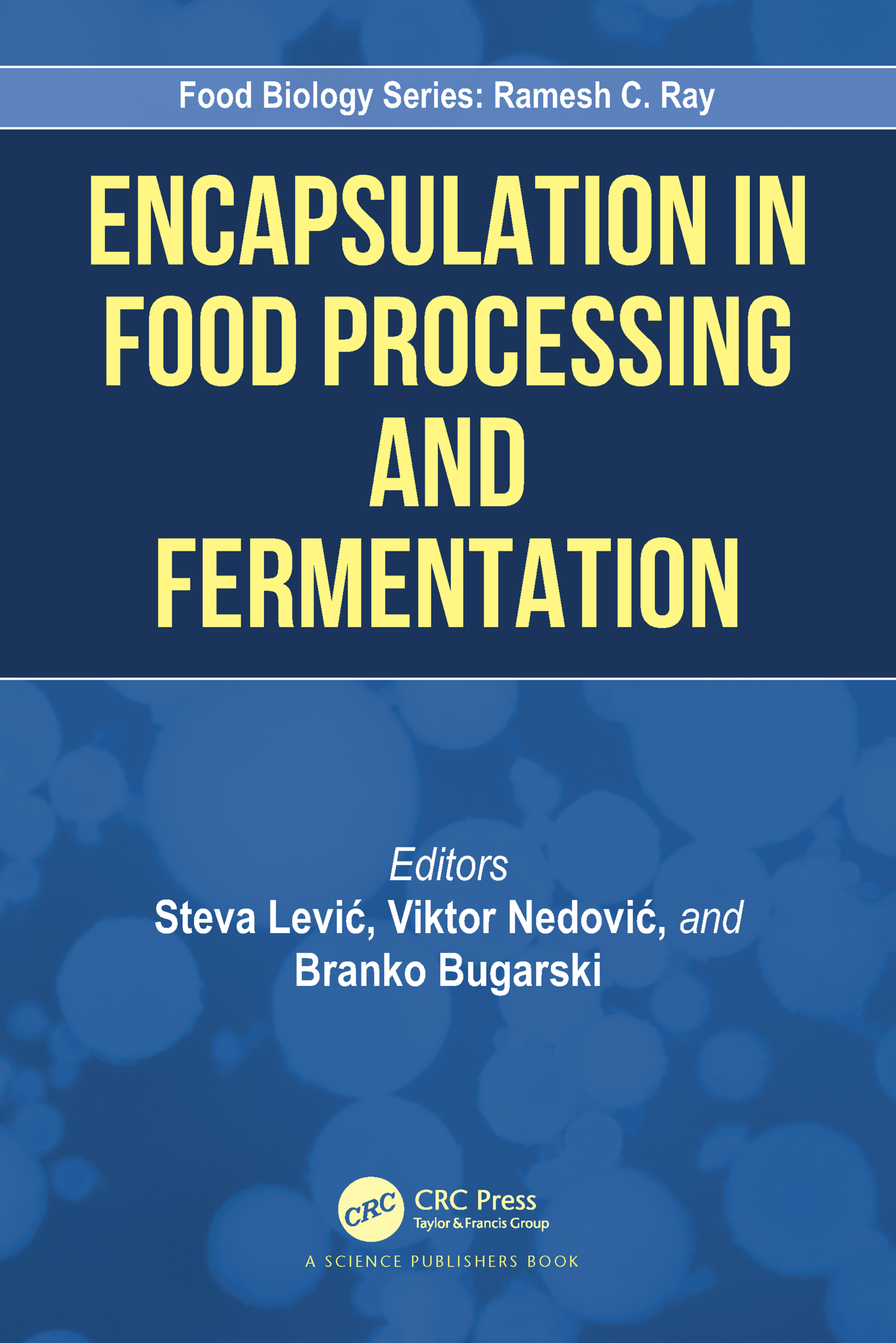
Steva Lević, Viktor Nedović, *and*
Branko Bugarski



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Preface to the Series

Food is the essential source of nutrients (such as carbohydrates, proteins, fats, vitamins, and minerals) for all living organisms to sustain life. A large part of daily human efforts is concentrated on food production, processing, packaging and marketing, product development, preservation, storage, and ensuring food safety and quality. It is obvious therefore, our food supply chain can contain microorganisms that interact with the food, thereby interfering in the ecology of food substrates. The microbe-food interaction can be mostly beneficial (as in the case of many fermented foods such as cheese, butter, sausage, etc.) or in some cases, it is detrimental (spoilage of food, mycotoxin, etc.). The *Food Biology* series aims at bringing all these aspects of microbe-food interactions in form of topical volumes, covering food microbiology, food mycology, biochemistry, microbial ecology, food biotechnology and bio-processing, new food product developments with microbial interventions, food nutrification with nutraceuticals, food authenticity, food origin traceability, and food science and technology. Special emphasis is laid on new molecular techniques relevant to food biology research or to monitoring and assessing food safety and quality, multiple hurdle food preservation techniques, as well as new interventions in biotechnological applications in food processing and development.

The series is broadly broken up into food fermentation, food safety and hygiene, food authenticity and traceability, microbial interventions in food bio-processing and food additive development, sensory science, molecular diagnostic methods in detecting food borne pathogens and food policy, etc. Leading international authorities with background in academia, research, industry and government have been drawn into the series either as authors or as editors. The series will be a useful reference resource base in food microbiology, biochemistry, biotechnology, food science and technology for researchers, teachers, students and food science and technology practitioners.

Ramesh C Ray
Series Editor

Preface

As a result of constant market demands and changes in consumers habits, the food industry has been engaged in a plethora of multidisciplinary researches in order to replace unhealthy and potentially dangerous ingredients with natural and more sustainable compounds; implementation of new strategies for improving the product shelf life; replacement of synthetic materials with biodegradable packaging films; use of the food industry byproducts as sources of new ingredients. Within all of these applications, encapsulation has been used as a tool for protecting of active compounds, providing controlled delivery and maintaining overall quality.

Encapsulation has been investigated as an alternative to conventional food processes, providing some benefits but also making the production process more complex and costlier. However, constant improvements and new carrier materials and encapsulation techniques offer critical impulse for establishing food products based on encapsulation technologies. Hence, it is not an exaggeration to claim that encapsulation technologies have become an integral part of the modern food sector. In order to fulfil very diverse demands, encapsulation technologies adopted new tools, materials and practices. The main directions in developing food encapsulation technologies are toward natural and renewable carrier materials, elimination or reduction of pollution and establishing more economically sustainable processes. However, the development and commercialization of encapsulation technologies have been a long process and included many trials and optimizations for any particular encapsulation techniques or carrier materials. For example, just twenty years ago, it was really challenging to purchase new encapsulation equipment, and prices were high as a result of the limited number of suppliers. Today, due to very intensive research and knowledge transfer, food companies and laboratories have numerous opportunities to obtain high-quality encapsulation equipment and materials. Thus, encapsulation is no longer unknown for the food sector, and consequently, numerous encapsulated food products are available for individual consumers and industry.

Considering the number of published papers and patents, it could be concluded that encapsulation is widely recognized and accepted in the food sector. Active compounds such as food flavors, colors, probiotic cells, mineral compounds, vegetable oils, antioxidants and many others are now available in encapsulated forms, optimized for a particular purpose. The main advantages of encapsulates, such as easy handling and protection of active compounds, controlled release, control of sensorial properties and specific marketing effects, are recognized and accepted as benefits for consumers and the overall product quality. On the other hand, encapsulation must be economically sustainable and should not become a burden to food production. Further, the health and ecological impact of encapsulated active compounds, especially those in the form of nanoparticles, are now a major topic in the scientific community.

The aim of this book is to provide an up-to-date overview of the main subjects related to encapsulation technologies in the food sector. Our authors present useful information for those who are in the field of encapsulation but also for those who are beginners in this field. We tried to summarize the main points in the development of encapsulated food compounds and to emphasize some limitations of encapsulation technology. The book contains chapters that review the newest encapsulation methods and carrier materials for the encapsulation of food compounds. Also, we describe general methods for the characterization of encapsulates as well as analytical methods for the analysis of the controlled release of active compounds. Further, the chapters related to specific topics such as the implementation of encapsulation technology in the production of beverages, dairy and meat products as well as the encapsulation of food supplements and plant extracts offer valuable results that may be of interest for academia but also for the broader community. Due to the significance of nanotechnology, we dedicated two chapters to this important topic.

The editors would like to thank and acknowledge all authors for their participating in the creation of this book in these challenging times. It was our great pleasure to be a part of the team, and we look forward to new joint projects in the future.

Steva Lević
Viktor Nedović
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Contents

CHAPTER

1

Introduction to Encapsulation Processes

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1. Introduction

In industry, particularly the food industry, encapsulation has been generally accepted as a beneficial technology that provides numerous advantages over conventional processes. Encapsulation is now considered as a solution to problems, such as degradation of active compounds, controlled delivery and protection of biocatalysts (Zuidam and Shimoni, 2010; Nedovic *et al*., 2011; Lević *et al*., 2016).

Encapsulation processes cover a large number of various active chemical compounds and biological systems, directly or indirectly used in the food industry. Encapsulation has been used for protection of various active food compounds:

- Food additives and ingredients (vitamins, minerals, flavor compounds, colorants, etc.);
- Microorganisms (live cells that produce various food products, such as beer, wine, fermented milk, etc.);
- Enzymes (used in specific processes that require enzyme encapsulation as a method for sustainable production);
- Other food-related active compounds (antimicrobial or antioxidant components of active packaging, etc).

Protection of an active compound is generally based on creating active protective layer(s) around it, making a physical and/or chemical barrier towards the environment. Usually such a system is defined as an *encapsulate* (Fig. 1). Encapsulates can be made into various shapes and sizes, depending on the applied encapsulation technique and properties of the active compound and carrier materials. Encapsulation systems are

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generally designed in accordance with specific applications and preferable properties of the final food product.

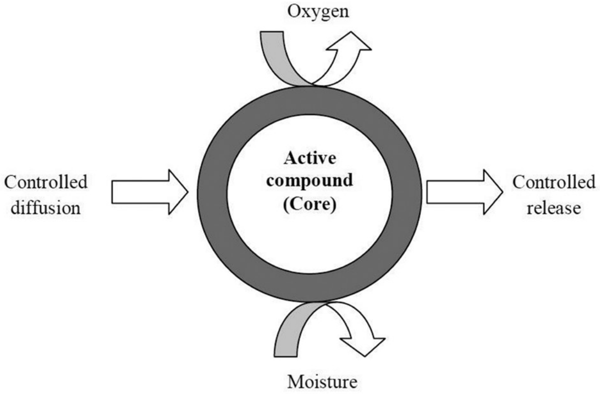

Fig. 1: General scheme of an encapsulate

For production of encapsulates with preferable properties, numerous techniques and industrial processes have been developed. These techniques are based on one or more phenomena that will shape the encapsulate into an adequate form and size (Nedović *et al*., 2001; Nedović *et al*., 2002; Thies, 2005; Prüsse *et al*., 2008; Zuidam and Shimoni, 2010). The size of encapsulates is a particularly important property regarding further usage in food products and food processes. According to Zuidam and Shimoni (2010) the size of encapsulates is mainly controlled by the applied encapsulation techniques. For example, spray-drying as the most frequently used industrial encapsulation technique provides encapsulates in the range 10-400μm; various extrusion techniques can be used for production of capsules with a size up to 5000μm or more.

The size of encapsulates has recently become even more important due to concerns regarding usage of nanoparticles in food industry and the necessary standards and safety procedures.

Carrier materials are of enormous importance in the development of adequate encapsulates as they primarily define the further fate of the encapsulated active compound. In this regard, in encapsulation processes, various materials have been adopted with more or less success. The two main groups of carrier materials are those from natural sources and synthetic materials. Further, the carrier materials can be divided into hydrophilic and hydrophobic groups. This is important since generally hydrophobic active compounds are encapsulated (coated) with a hydrophilic carrier and vice versa. The main groups of carrier materials used in food applications are carbohydrates (sugars, starch, cellulose, gums, dextrins), proteins (soy proteins, corn proteins, gelatin, casein, whey protein isolates), lipids (hard fat, fatty acids, waxes, phospholipids), synthetic polymers and modified natural carrier materials (Sobel *et al*., 2014).

The selection of appropriate carrier materials in the encapsulation processes remains the crucial point of every encapsulation since there is no universal carrier material that can fulfill all requirements. Also, many carrier materials exhibit some level of biological activity; hence there is an interest in using such materials in food-related encapsulations.

2. The Goals of Encapsulation in Food Technology

The main goals of encapsulation in food processes differ between various applications and types of active compounds, but generally, there are several main goals of encapsulation:

- Protection of an active compound against unfavorable environmental conditions;
- Controlled delivery of an active compound;
- Improved handling of an active compound;
- Masking of an unpleasant odor and taste associated with an active compound.

The list is a simplified presentation of encapsulation possibilities that may also include reuse of encapsulated/immobilized biocatalysts and creation of certain visual and textural effects upon consumption (Zuidam and Shimoni, 2010).

Benefits of introducing encapsulation into production processes are potentially significant. The main role of encapsulation in food industry remains protection of an active compound against the negative influence of environmental factors. Among these factors, high temperatures and oxygen-related degradation processes are main reasons for the loss of an active compound. For example, improving thermal stability of aromas is critical in many food thermal processes. Hence, protection of thermal-sensitive compounds by encapsulation is sometimes the only solution for their application in food (Kayaci and Uyar, 2012). Besides protection, encapsulation simultaneously could be used for production of encapsulates that transform active compounds into easier-to-use forms. Good examples are liquid aroma compounds for which easy handling and thermal protection could be achieved in a single encapsulation process (Lević *et al.*, 2015).

Controlled delivery (or controlled release) of active compound is one of the main benefits of encapsulation and one of the main reasons for the great progress of encapsulation in recent decades. The most important step in the development of a controlled delivery system is the recognition of place of delivery (i.e. place of release) of the active compound and consequently selection of an adequate carrier material and encapsulation technique. According to Huynh and Lee (2014) the mechanisms of active compound release depend on physical and chemical properties of carrier materials, but can also be considered a stimuli-controlled process. Therefore, the projection of active compound controlled release is critical for successful application of encapsulate in real food products. For this purpose, *in vitro* tests have been developed in order to simulate specific conditions of human gastrointestinal system (Minekus *et al.*, 2014). In the food industry, controlled release encapsulation systems are usually implemented in food products as carrier systems for probiotics (Burgain *et al.*, 2011; Dimitrellou *et al.*, 2016; Dimitrellou *et al.*, 2019). Other applications include thermal protection (and release) and facilitating handling of additives, such as flavors (Milanovic *et al.*, 2010; Kayaci and Uyar, 2012; Lević *et al.*, 2015), edible oils (Beindorff and Zuidam, 2010; Stajić *et al.*, 2014; Stajić *et al.*, 2018), micronutrients (Zimmermann and Windhab, 2010), etc.

On the other hand, the goals of biocatalyst encapsulation are mainly related to the protection against stress conditions, such as extreme temperature and pH and inhibitory effects of substrate or products. Also, encapsulation of biocatalysts is performed in order to ensure reuse of expensive compounds (i.e. biocatalysts), such as enzymes, or in continuous bioreactor processes to prevent or minimize loss of biocatalysts as a result of intense substrate flow (Lević *et al*., 2016).

Introduction of encapsulation into the food production process is a complex task and requires knowledge of technological details of encapsulation, management of processes as well as precise economic analysis. For example, sometimes encapsulation is the only way to overcome technical problems, such as inhibition of a biocatalyst during a biotechnological process (Lalou *et al*., 2013), or to contain active compound that in the free form may be lost during food processing (Stajić *et al*., 2014; Stajić *et al*., 2018). However, encapsulation is sometimes a complex process and a broad analysis is needed prior to encapsulation of active compound and introduction into a food product. Moreover, the fate of encapsulated active compound must be also projected as well as its storage stability, storage conditions and package options. For these reasons, establishing of food encapsulation processes on an industrial level is a multidisciplinary task as well as a significant financial effort.

3. Historical Background of Encapsulation

Historically, encapsulation processes are relatively well established in industry and science. The successful applications of encapsulation processes could be found in various industrial sectors and usually depend on current needs of the market.

There is a general consensus among the authors that the first encapsulation technologies started with first patent applications and reported research in the 19th century (Sobel *et al*., 2014; Tucker *et al*., 2012). This is not a surprise considering the fact that at the same time many countries entered the Industrial Revolution, a period of history with never-before-seen level of inventions and ideas.

The practices that involve some form of protection of active ingredients or biocatalysts are recorded through the history, emphasizing the needs and problems of a particular epoch. A good example for this is the solution for production of copy paper that comes from the 1930s. The basis for this invention was encapsulated color that was released as result of pressure during writing (Fanger, 1974). The needs sometimes dictate the unusual solutions and sometimes could be considered as true masterpieces, and encapsulation technologies are not exempt from this. A good example is the development of coacervates that were first used in the paper industry (Sobel *et al*., 2014), but later were frequently studied as tools for encapsulation of food active compounds (Zuidam and Shimoni, 2010). Some important points in the development of encapsulation processes are presented in Table 1.

Development of some encapsulation processes is really an interesting story. For example, production of materials consisting of nanofibers has a long history. The first results of using electrostatic force for formation of nanomaterials (i.e. by using an electrospinning configuration) were published (in the form of a patent) in 1900. Further development led to establishment of the first factories for production of electrospun materials for various applications (Tucker *et al*., 2012). This inevitably led to adoption of electrospun fibers in food-related studies; namely, electrospun fibers containing natural compounds could be a basis for production of new types of packaging materials, optimized for reduction of chemical food preservatives in food products (Vafania *et al*., 2019). This approach in encapsulation of active ingredients is

Table 1: The Historical Development of Encapsulation Processes

Year	Encapsulation Process	Application	Comments	References
1872	The first spray drying patent.	Milk powder production.	Proposed technique was also evaluated for drying of dextrin, starches and gelatin.	Sobel *et al*., 2014
1900	The first electrospinning patent.	Model system for electrospinning (nitrocellulose).	Coaxial needle system as support for electrospinning process.	Tucker *et al*., 2012
1938	Electrospun fibers as basis for gas masks' filters.	Basis for cellulose acetate filters in gas masks.	Known as 'Petryanov filters', they are basis for filters in the nuclear industry.	Tucker *et al*., 2012
1957	Development of the technique for oil encapsulation that later will become known as coacervation.	Basis for first generation of carbonless paper.	The new technique of encapsulation will become an impulse for development of new encapsulation techniques.	Sobel *et al*., 2014; Zuidam and Shimoni, 2010
1958	Electrostatic droplet extrusion.	Preparation of emulsions.	One of the first applications of electrostatic force for generation of uniform droplets.	Kim *et al*., 2019
1960s	Established the basis for liposome encapsulation.	Basic studies on chemistry of new structures that will lead to further development and applications of liposome technology.	The studies were oriented toward analysis of surface and functional properties of liposome.	Sobel *et al*. (2014)
1990	Development of microfluidic devices.	The new process for generation of cells encapsulates.	The development of microfluidic devices provided basis for cells encapsulation and study in many area of biotechnology and medicine.	Kim *et al*., 2019
1980s and 1990s	Development of numerous encapsulates based on cyclodextrins as carrier materials.	These processes are commonly known as inclusion (or molecular inclusion).	These processes are important for food industry due to the fact that cyclodextrins may provide efficient encapsulation and controlled release of aromas.	Crini (2014)
1980s and 1990s	Many patent applications regarding protection of food compounds.	The focus of these patents was mostly on protection of volatile compounds.		Sobel *et al*., 2014

promising since various carrier materials are suitable for processing into the form of fine fibers and films (Salević *et al.*, 2019; Dehcheshmeh and Fathi, 2019).

Besides encapsulation techniques, at the same time numerous carrier materials have been developed too, providing possibilities for development of new encapsulation techniques. A good example for this is development of cyclodextrins. The first descriptions of cyclodextrins in literature could be found in 1891, however, the development of encapsulates using these molecules with exceptional properties as carriers did not start until their structure was closely studied. So, as development and availability of analytical methods progressed, more data about cyclodextrins were collected, providing the basis for publishing numerous papers and patents targeting molecular inclusion of various compounds into cyclodextrins structure. Today, cyclodextrins are frequently studied as carriers for encapsulation of active compounds in various industrial applications (Crini, 2014).

4. Encapsulation: State-of-the-Art

Encapsulation is gaining attention in many technological processes and research. Currently, encapsulation systems are mainly applied in pharmacy (medicine) and the food sector. The relevance of encapsulation in the food sector could be measured by using available scientific databases. According to Web of Science and Scopus (accessed on 11 November, 2020), and using ‘encapsulation, food’ as search criteria, the number of published papers is above 4,000 (Fig. 2a).

Moreover, the success of encapsulation could be also measured by the sum of citations by year (Fig. 2b). Regarding global position of encapsulation in the food sector and science, the main national contributors are China, USA, Brazil, Iran, India, Spain, etc. (Scopus database, accessed on 11 November, 2020; keywords ‘encapsulation, food’). As can be seen, this field is dominated by the large world economies and countries with a huge number of research centers. However, due to increased knowledge transfers and more available scientific data, more countries and research facilities have been included in encapsulation of food components and their evaluation.

At the industrial level, spray drying remains the most frequently used method for production of food encapsulates. The main advantage of spray-drying is rapid solvent evaporation and encapsulate formation in a continuous operation (Zuidam

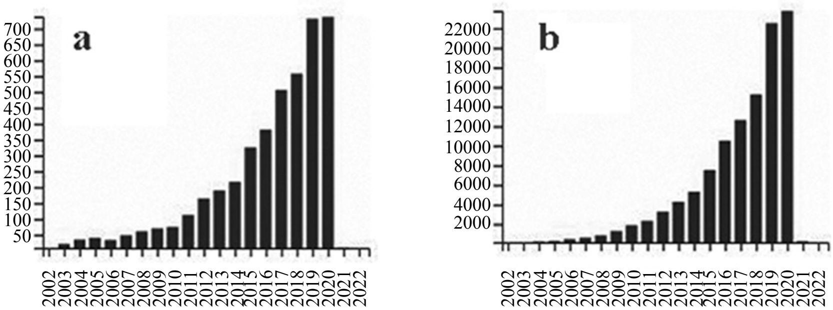

Fig. 2: Total publications by year (a) and sum of times cited by year (b) since 2002 obtained from online database Web of Science using ‘encapsulation, food’ for search (accessed on 11 November, 2020)

solvent evaporation and encapsulate formation in a continuous operation (Zuidam and Shimoni, 2010). However, to produce encapsulates with satisfactory properties, relatively high concentrations of carrier materials are required (Kalušević *et al.*, 2017b). Further improvements of spray-drying technology could be in the direction of new dispersion systems and solvent evaporation under an inert atmosphere. Liquid dispersion into small droplets (of few microns) using modified spray dryers has been recently adopted as a suitable method for production of nanoparticles and nanoencapsulates. This method is suitable for encapsulation of plant extracts (Del Gaudio *et al.*, 2017, Kyriakoudi and Tsimidou, 2018) or food ingredients, such as salts (Moncada *et al.*, 2015). Besides improved bioavailability, nano spray-drying provides particles that could be more easily dissolved and hence leads to increase in taste perception. Consequently, this could lead to a decrease in concentration of some food ingredients which are generally recognized as causes of some modern human diseases (Moncada *et al.*, 2015).

Spray-drying in an inert atmosphere is necessary when flammable solvents are used for extraction of active compounds. To proceed further with conventional spray drying (i.e. using hot air as drying medium), flammable solvents must be removed by evaporation. However, this step could be avoided by using an inert gas as the drying medium (e.g. nitrogen), which lowers the possibility for explosion during spray-drying. Also, this approach is suitable for application of conventional carrier materials used in spray-drying (Vázquez-León *et al.*, 2020). Inert gases used in spray-drying may cause changes in morphology and structural properties of obtained powders. Depending on the applied gas, a more amorphous powder can be obtained (Islam and Langrish, 2010). Besides solvent regeneration and improved safety, usage of closed-loop spray-drying has become a promising solution in ongoing energy regulations and need for reduction of pollution and establishing of more sustainable drying processes (Moejes *et al.*, 2018).

Other encapsulation techniques may also cause changes in the crystal structure of active compounds, consequently leading to modification of some important properties, such as melting point. This could be explained by the influence of carrier materials and specific conditions during encapsulate formation (Lević *et al.*, 2014).

Emulsions are important systems for the food sector and many food products depend on emulsion stability. Since stability of emulsions is affected by numerous factors, production of successful emulsion-based products is challenging (Dickinson, 2010). Emulsions are very suitable delivery systems for controlled release of food flavors (Mao *et al.*, 2017), some nutritionally valuable compounds (Matos *et al.*, 2018) and probiotics (Su *et al.*, 2021). Although high-energy processes are still the main methods for emulsions preparation, procedures that involve low-energy consumption have become more popular, especially when sensitive active compounds are included in formulations. Santana *et al.* (2013) reviewed these methods in more detail, pointing out the importance of surfactant properties for formation of stable emulsion using low-energy emulsification processes. Also, membrane emulsification is another approach that could be an alternative for conventional emulsions and particles preparation. The emulsion is formed by passing the dispersed phase into the continuous phase, using specially designed membranes and operating under low pressure. These processes are especially suitable for production of encapsulated food active compounds in the form of liposomes (Charcosset, 2009).

Besides food additives, the second important area for application of encapsulation in food production is protection of biocatalysts. The goal of biocatalysts encapsulation/immobilization is usually oriented toward reuse of enzyme/cells in consecutive production cycles, with additional protection against environmental stress (i.e. high concentration of nutritional medium or inhibitory effects of products). In the case of expensive biocatalysts, encapsulation/immobilization is a solution for easy manipulation, especially in the processes that use enzymes and where, due to their small sizes, it is difficult to separate them and reuse in the next cycle. A good example of a highly successful application of immobilized biocatalysts is the production of high fructose syrup using immobilized enzymes (Lević *et al.*, 2016).

Conventional encapsulation of biocatalysts is usually based on application of adsorption methods, where cells or enzymes are linked to carrier's surface via specific physical/chemical interactions or biocatalysts are entrapped into the structure (i.e. pore) of carrier materials (Verbelen *et al.*, 2010; Costa *et al.*, 2004). The efficiency of adsorption depends on many factors, such as the type of biocatalyst (i.e. enzyme or cells), structure and chemical property of materials, porosity, etc. (Costa *et al.*, 2004; Reddy *et al.*, 2011). Other suitable methods of biocatalyst encapsulation usually include the application of some porous matrix that covers the biocatalyst and provides satisfactory diffusion properties regarding nutrients and reaction products. For this purpose, numerous porous carriers based on natural or synthetic materials have been developed. Their shape and size could be regulated in order to obtain carriers with optimal performance. Also, such carriers must be optimized for application under specific bioreactor conditions (Verbelen *et al.*, 2010; Lević *et al.*, 2016).

The main problem regarding introduction of immobilized biocatalysts in production of food products is the potential negative influence of the encapsulation procedure, particularly the influence of carrier materials on sensorial properties of the final products. To avoid this, research is focused on inert carrier materials, such as natural polysaccharides (e.g. alginate, chitosan and pectin), proteins (e.g. gelatin, collagen) or synthetic polymers, such as polyvinylalcohol (PVA) (Verbelen *et al.*, 2010). Inorganic carrier materials are also suitable for this purpose, especially due to their high chemical, thermal and mechanical stability. For example, special glasses (Marchis *et al.*, 2012) or specially synthesized zeolites (Kumari *et al.*, 2015) may be used for enzyme immobilization. In order to reduce the application of synthetic carrier materials, some studies have been focused on usage of food processed byproducts or low processed natural carriers for encapsulation/immobilization of enzymes and cells. Some examples include use of sugarcane pieces (Reddy *et al.*, 2011), watermelon pieces (Reddy *et al.*, 2008) or pear pieces (Mallios *et al.*, 2004) for yeast cells immobilization. Other renewable resources of carrier materials could be obtained as results of microbial metabolic processes. For example, bacterial cellulose could be used as solid support for yeast cells immobilization (Ton and Le, 2011). Also, active cells, such as yeast can be co-immobilized using filamentous fungi, providing promising support for wine fermentation (Puig-Pujol *et al.*, 2013).

Another interesting concept in cell encapsulation is the creation of protective organic or inorganic layers around individual cells. This concept is described in the literature as 'cyborg cells' (Fakhrullin *et al.*, 2012). This approach offers numerous possibilities, especially for cell protection in those processes that require application of stressful environmental conditions and where conventional encapsulation procedures and materials cannot provide an adequate barrier.

The biotechnological processes that involve use of live cells as biocatalysts are influenced by numerous factors that may make these processes economically unsustainable; namely, cells usually require complex substrates for maintaining cellular functions. During the process, substrate is partially used by the cells for production of products and one significant amount is utilized for the cell's metabolism. Also, cells may undergo the change in its genetic material (i.e. DNA), which further may result in decrease of productivity. The potential solution for this is usage of enzymes. However, complex cellular processes require numerous enzymes and their balanced activity. To overcome these problems and at the same time to exclude the cells from the process, enzymes involved in production of targeted compound are joined by encapsulation in the form of an enzyme cascade. Patterson *et al.* (2014) described one such system where several enzymes were encapsulated into small particles, mimicking cellular conditions. According to Chen *et al.* (2018), an enzyme cascade could be constructed into the form of nano-bioreactors, consisting of several necessary enzymes involved in the desired transformation of substrate. Besides, such an approach may lead to better protection of enzymes and even partial suppression of the formation of undesirable products.

Although the current application of nano-systems in the food sector is limited, further development and potential use are to be expected. Currently, these systems are mainly oriented towards encapsulation/immobilization of enzymes. Inorganic materials based on silica particles, combined with adequate polymers may result in stable enzymatic carriers. Also, materials based on carbon nanotubes are promising enzyme carriers in development of biosensors. Another major group of nano-systems with huge potential in enzyme encapsulation are magnetic nano-particles. The major advantage of magnetic carriers is their easy recovery and control inside bioreactors using a magnetic field. This opens numerous possibilities for process control as well as for a new design of bioreactors (Lević *et al.*, 2016). Also, magnetized cells could be applied with the same goals as enzymes. Cell magnetization could be performed by specially designed magnetic nanoparticles that are positive charged and easily applied on to negative charged cells. Such cells showed satisfactory results when applied in fermentation procedures (Dušak *et al.*, 2016).

5. Health and Environmental Impact of Encapsulation Processes

As pointed out above, the protection of active compounds against degradation or reduction of their nutritional value is one of the main reasons for the introduction of encapsulation in the food sector. Also, by using encapsulation, problems related to dosage and standardization of health beneficial compounds can be solved. According to available literature data, the main application of encapsulation in the food sector is protection of probiotic cells and their controlled delivery. Besides probiotics, one of the most frequently studied group of encapsulated active compounds are plant-based antioxidants (Belščak-Cvitanović *et al.*, 2011; Belščak-Cvitanović *et al.*, 2015; Kalušević *et al.*, 2017a,b; Estakhr *et al.*, 2020). This is not a surprise since plants are a traditional source of nutritionally valuable chemicals, with a generally well-known composition and defined safety risks. However, some plant products, such as some extracts or essential oils have strong (usually negative) sensorial effects on consumers. To overcome these problems, encapsulation could be used for masking the unpleasant taste and to control the sensorial profile of products. Plant essential oils are well known for their medicinal effects, but due to the intense fragrance they require masking and controlled delivery in order to be incorporated into foods. Although essential oils are complex mixtures of various volatile compounds, their encapsulation is relatively simple and could be based on food-grade carriers suitable for incorporation into food products (Yilmaztekin *et al.*, 2019).

Encapsulation could be also important as a tool for prevention of undesirable chemical reactions and formation of harmful compounds. For example, reducing the formation of Maillard reaction products in thermally-processed foods could be achieved by encapsulation of sodium chloride prior to product baking. The prevention of Na^+ cations contact with precursors of Maillard reaction products was found to be critical in reduction of 5-hydroxymethylfurfural and acrylamide content in baked products. Moreover, encapsulation offers possibilities for use of various lipid-based carriers for this purpose (Fiore *et al.*, 2012).

The food industry produces a significant amount of waste that needs to be processed. This is especially important in the case of wastewater generated during food production that could contain a significant concentration of organic materials. The other problem is that the total amount of wastewater generated during food processing can be significant and could be a large financial burden. Hence, intensification of water treatment processes using encapsulated biocatalysts has attracted great attention in the recent decades. Some specific applications of immobilized cells and enzyme technology for wastewater treatments could be removal of cyanide (Chen *et al.*, 2007), endocrine-disrupting chemicals (Maryšková *et al.*, 2020), nitrogen (Dolejš *et al.*, 2019) and pesticides (Lin *et al.*, 2020).

Besides the numerous advantages of encapsulation systems applied in water and waste treatment, we should mention some potential problems related to encapsulation and their potential influence on ecosystems.

There are two major issues that need to be addressed regarding encapsulation processes and ecology. First, the applied carrier materials and active ingredients that form capsules should be from renewable resources. This requirement arises from recent strategies adopted by many countries with the goal of reducing emissions of harmful compounds and making production more energy efficient.

The second issue is closely related to concerns that many materials (mainly synthetic) formed in various industrial processes remain in ecosystems for prolonged periods of time. Browne *et al.* (2011) analyzed pollution by microplastic and concluded that plastic materials tend to accumulate in marine ecosystems and could potentially cause health problems for aquatic organisms. This also raises the question of encapsulates' fate if they are released in the environment without previous knowledge of potential threats to environment.

The food industry usually uses widely approved components that are recognized as safe for nutritional applications. However, in the last several decades, numerous additives and food-related materials have been banned or their usage was significantly restricted. Introduction of new forms of additives and active compounds, i.e. in the form of encapsulates, could become a challenge for existing waste-treatment facilities and processes. Also, biodegradation of encapsulated materials must be thoroughly investigated in order to prevent future problems, such as those with generated microplastics.

6. Economy of Encapsulation Processes

As pointed out above, encapsulation provides numerous advantages and opens new possibilities for development of new food products. However, according to Zuidam and Shimoni (2010) the main obstacles that must be taken into consideration during planning of food encapsulation processes are investment costs; specially introduction of encapsulation into production could be a costly operation which requires a new encapsulation unit (i.e. production line) or use of commercially available encapsulated active compounds. The first approach is more complex and during calculation of investment costs, the following economical prices must be taken into consideration (Dimitrellou *et al.*, 2019):

- Variable costs (cost of carrier materials and active compound, energy costs, etc.);
- Fixed costs (these costs may include depreciation, interest, etc.).

These are the basic inputs for calculating the economical sustainability of the encapsulation process. Further, the structure of costs depends on the applied encapsulation process and all preparation and finishing steps after the main operation (i.e. encapsulation). Figure 3 summarizes the steps in the production of encapsulates that contain living cells. This scheme is, in our opinion, the most complex in the production of encapsulate and includes many steps that critically influence the properties of encapsulated cells as well as economical sustainability of encapsulation.

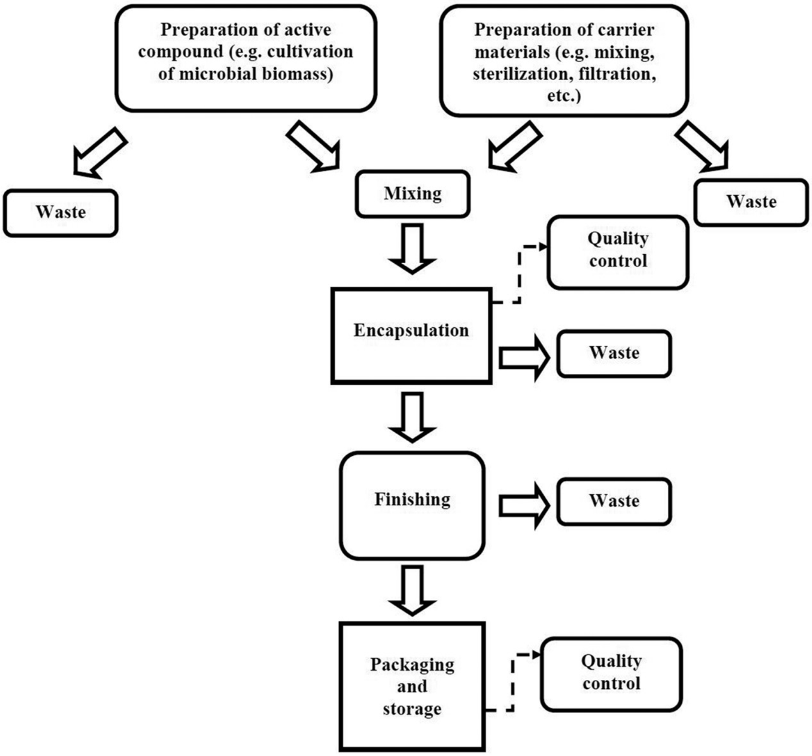

Fig. 3: Schematic representation of main phases of encapsulation process for production of cells encapsulates

Further, the scheme doesn't include steps like water preparation, storage of materials, manipulative operations, and sanitation. A significant problem is the management of waste created in almost all the main production steps. The structure of waste depends on the encapsulation procedure, but generally may include wastewater, dust, solid particles, biomass, etc. Depending on the structure and local environmental laws, management of the waste could be a costly operation that must be considered during calculation of the encapsulation investment.

Dimitrellou *et al.* (2019) analyzed the economic sustainability of introducing an encapsulation process into the production of fermented milk. The analysis, from the technical point of view, was based on usage of probiotic cells encapsulated into Ca-alginate capsules. Also, the analysis included the projections of fermented milk production capacity and the prices in the regions of interest. According to the same authors, the economic analysis showed that over a period of eight years, and by managing dairy capacities between 70-100 per cent, it is possible to maintain the production of encapsulates and consequently fermented milk, using encapsulated probiotic cells.

Various factors may influence the price of encapsulation process. Some of these factors are costs of drying operations, particles size, type of carrier materials, types of industrial process (i.e. pharmaceutical industry or other industrial branches), etc. On these topics, we recommend the chapter published by Veršič (2014), where many useful data regarding encapsulation process costs are available.

Other limitations regarding economical sustainability of food encapsulation processes may be expressed by the complex influence of climate and political factors, as well as local regulation. The first two factors are closely connected and could have devastating effects on product supply. These factors may influence supply of active ingredients as well as carrier materials. One good example for the complex influence of climate and political factors on encapsulation is availability of gum arabic (gum acacia). According to Wandrey *et al.* (2010) gum arabic is an excellent carrier material that has numerous advantages, such as preferable viscosity (even at high concentrations), good protection of active ingredients against oxidation and negative influence of moisture from the air. For example, gum arabic was found to be an excellent carrier material for encapsulation of plant active compounds via spray-drying (Kalušević *et al.*, 2017a,b). However, the main production region for gum arabica, i.e. for the acacia plant from which this material is obtained is located in Africa, more precisely in the ecoclimatic region of Sahel (Wandrey *et al.*, 2010). The acacia tree is generally more resistant toward arid climate compared to other commercially important tree species of Sahel region (Gonzalez *et al.*, 2012). However, overall climate changes in the region may be sources of further conflicts that could disrupt supply of gum arabica (Arcanjo, 2019). Also, disruption of supply of natural active compounds may consequently lead to a blockade of the whole production process of encapsulation. Hence, alternatives should be closely analyzed and included in the production plans. Some potential strategies regarding potential substitution of active compounds and carrier materials are shown in Table 2.

Another potential alternative to conventional sources of active compounds and carrier materials could be plant cells cultivated in bioreactors. By using this approach many problems related to collected or cultivated plants, such as destruction of environment, pollution, influence of climate and climate changes and political insecurity could be potentially solved. Currently, there are reports of plant cells cultivation for

Table 2: Some Examples of Potential Substitutions of Active Compounds and Carrier Material in the Case of Disruption of Supply Chain

	Used material	Alternative	Comments	References
Carrier materials	Gum arabica	Maltodextrin, skimmed milk	All three materials showed good properties in process of spray drying of plant extracts.	Kalušević *et al*., 2017a,b
	Alginate (or polyvinyl alcohol)	Combined carrier materials for cell immobilization.	Both the materials mix well and can protect cells. The materials are templates and their share can be changed.	Bezbradica *et al*., 2004; Radosavljević *et al*., 2020
	Natural waxes	Various fatty acids	Results depend on potential applications. All materials have good barrier properties but depend on thermal properties of carrier materials.	Fiore *et al*., 2012
	Native starch	Modified starches	Based on their properties, modified starches could improve properties of encapsulates.	Zhu, 2017
Active compounds	Carotenoids	Alternatives include various plants sources such as red pepper, carrot, some vegetables processing waste.	Regarding their chemical properties, plant carotenoids from various sources could be encapsulated using similar processes and carrier materials.	Vulić *et al*. (2019) ; Šeregelj *et al*., 2018
	Resveratrol	Acetylresveratrol	Acetylresveratrol is more stable and bioactive compared to resveratrol.	Su *et al*., 2020
	Vanillin	Ethyl vanillin	Natural vanillin could be replaced even in the encapsulated forms with synthetic ethyl vanillin.	Lević *et al*., 2014
	Edible oils	Other, more appropriate and more available vegetable oils, but with similar bioactive properties.	Some oils are more acceptable regarding sensorial properties or they are more available than others.	Stajić *et al*., 2014, 2018

production of proteins (Dörnenburg, 2010), plants secondary metabolites (Marchev and Georgiev, 2020) and microalgae biomass and active compounds (Xu *et al.*, 2009).

Encapsulation in biotechnology offers new possibilities for investors and academia to develop new useful products through cooperation and knowledge transfer. However, encapsulation should first overcome one barrier, namely, according to Veršič (2014), the perception of encapsulation is still mainly focused on it as a service. However, in recent years, there is a trend for new encapsulated products which may change perception on encapsulation and open new possibilities for industry and research.

7. Conclusion and Future Perspectives

In the past several decades, encapsulation has shown its potential to solve many problems related to the preservation of food active compounds, controlled delivery, food packaging, etc. It can be expected that encapsulation will remain a powerful tool in many aspects of food industry, providing a basis for more efficient and innovative processes and products. Also, encapsulation could find place in the newly established concepts and strategies, such as personalized nutrition and the circular economy.

Personalized nutrition is a concept where real nutritional needs are managed in accordance with each person. Under this concept, each individual is analyzed regarding his/her own nutritional needs, especially considering personal health condition, genetics, lifestyle, etc. (Betts and Gonzalez, 2016; McClements, 2020). Encapsulation could be used in personalized nutrition where specific controlled delivery is required for both pure or a mixture of active compounds.

The other important aspect of modern society strongly connected to the food industry is a circular economy. A circular economy is a concept of sustainable use of natural resources via recycling/reuse of available resources and reduction in applying new (natural) resources, reduction or elimination of waste and pollution. One of the main concerns regarding this concept is plastic pollution, particularly plastics that originate from food packaging (Black *et al.*, 2019). Encapsulation has been recognized as a suitable solution in production of active packaging systems, where active ingredients are encapsulated and incorporated into packaging material (preferably natural or biodegradable), and provide a new line in defense of food products.

We hope that readers of this book will find the contents interesting and informative. The authors of the book's chapters cover all major aspects of encapsulation processes in food industry with a critical analysis of available literature data. Editors are grateful to authors for their time and energy invested in this book.

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References

Arcanjo, M. (2019). Risk and Resilience: Climate Change and Instability in the Sahel, Climate Institute, New York, US; http://climate.org/risk-and-resilience-climate-change-and-instability-in-the-sahel/.

Beindorff, C.M. and Zuidam, N.J. (2010). Microencapsulation of fish oil, pp. 161-186. *In*: N.J. Zuidam and V. Nedovic (Eds.), *Encapsulation Technologies for Food Active Ingredients and Food Processing*, Springer, Dordrecht.

Belščak-Cvitanović, A., Stojanović, R., Manojlović, V., Komes, D., Juranović Cindrić, I., Nedović, V. and Bugarski, B. (2011). Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate-chitosan system enhanced with ascorbic acid by electrostatic extrusion, *Food Research International*, 44: 1094-1101.

Belščak-Cvitanović, A., Lević, S., Kalušević, A., Špoljarić, I., Đorđević, V., Komes, D., Mršić, G. and Nedović, V. (2015). Efficiency assessment of natural biopolymers as encapsulants of green tea (*Camellia sinensis* L.) bioactive compounds by spray-drying, *Food Bioprocess Technology*, 8(12): 2444-2460.

Betts, J.A. and Gonzalez, J.T. (2016). Personalized nutrition: What makes you so special? *Nutrition Bulletin*, 41: 353-359.

Bezbradica, D., Matić, G., Nedović, V., Čukalović-Leskošek, I. and Bugarski, B. (2004). Immobilization of brewing yeast in PVA/alginate microbeads using electrostatic droplet generation, *Chemical Industry*, 58: 118-120.

Black, J.E., Kopke, K. and O'Mahony, C. (2019). Towards a circular economy: Using stakeholder subjectivity to identify priorities, consensus, and conflict in the Irish EPS/XPS market, *Sustainability*, 11: 6834.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T. and Thompson, R. (2011). Accumulation of microplastic on shorelines woldwide: Sources and sinks, *Environmental Science & Technology*, 45: 9175-9179.

Burgain, J., Gaiani, C., Linder, M. and Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications, *Journal of Food Engineering*, 104: 467-483.

Charcosset, C. (2009). Preparation of emulsions and particles by membrane emulsification for the food processing industry, *Journal of Food Engineering*, 92: 241-249.

Chen, C.Y., Kao, C.M., Chen, S.C., Chien, H.Y. and Lin, C.E. (2007). Application of immobilized cells to the treatment of cyanide wastewater, *Water Science & Technology*, **56**(7): 99-107.

Chen, W.H., Vázquez-González, M., Zoabi, A., Abu-Reziq, R. and Itamar Willner, I. (2018). Biocatalytic cascades driven by enzymes encapsulated in metal-organic framework nanoparticles, *Nature Catalysis*, 1: 689-695.

Costa, S.A., Azevedo, H.S. and Reis, R.L. (2004). Enzyme immobilization in biodegradable polymers for biomedical applications, pp. 301-324. *In*: R.L. Reis and S.J. Román (Eds.), *Biodegradable Systems in Tissue Engineering and Regenerative Medicine*, CRC Press, Boca Raton, Florida.

Crini, G. (2014). Review: A history of cyclodextrins, *Chemical Reviews*, **114**(21): 10940-10975.

Dehcheshmeh, M.A. and Fathi, M. (2019). Production of core-shell nanofibers from zein and tragacanth for encapsulation of saffron extract, *International Journal of Biological Macromolecules*, 122: 272-279.

Del Gaudio, P., Sansone, F., Mencherini, T., De Cicco, F., Russo, P. and Aquino, R.P. (2017). Nanospray drying as a novel tool to improve technological properties of soy isoflavone extracts, *Planta Medica*, **83**(05): 426-433.

Dickinson, E. (2010). Food emulsions and foams: Stabilization by particles, *Current Opinion in Colloid & Interface Science*, 15: 40-49.

Dimitrellou, D., Kandylis, P., Lević, S., Petrović, T., Ivanović, S., Nedović, V. and Kourkoutas, Y. (2019). Encapsulation of *Lactobacillus casei* ATCC 393 in alginate capsules for probiotic fermented milk production, *LWT – Food Science and Technology*, 116: 108501.

Dimitrellou, D., Kandylis, P., Petrović, T., Dimitrijević-Branković, S., Lević, S., Nedović, V. and Kourkoutas, Y. (2016). Survival of spray dried microencapsulated *Lactobacillus casei* ATCC 393 in simulated gastrointestinal conditions and fermented milk, *LWT – Food Science and Technology*, 71: 169-174.

Dolejš, I., Stloukal, R., Rosenberg, M. and Rebroš, M. (2019). Nitrogen removal by co-immobilized anammox and ammonia-oxidizing bacteria in wastewater treatment, *Catalysts*, 9: 523.

Dörnenburg, H. (2010). Cyclotide synthesis and supply: From plant to bioprocess, *Biopolymers*, **94**(5): 602-610.

Dušak, P., Benčina, M., Turk, M., Bavčar, D., Košmerl, T., Berovič, M. and Makovec, D. (2016). Application of magneto-responsive *Oenococcus oeni* for the malolactic fermentation in wine, *Biochemical Engineering Journal*, 110: 134-142.

Estakhr, P., Tavakoli, J., Beigmohammadi, F., Alaei, S. and Mousavi Khaneghah, A. (2020). Incorporation of the nanoencapsulated polyphenolic extract of Ferula persica into soybean oil: Assessment of oil oxidative stability, *Food Science & Nutrition*, 00: 1-10.

Fakhrullin, R.F., Zamaleeva, A.I., Minullina, R.T., Konnova, S.A. and Paunov, V.N. (2012). Cyborg cells: Functionalization of living cells with polymers and nanomaterials, *Chemical Society Reviews*, 41: 4189-4206.

Fanger, G.O. (1974). Microencapsulation: A brief history and introduction, pp. 1-20. *In:* J.E. Vandegaer (Ed.), *Microencapsulation*, Springer, Boston, MA.

Fiore, A., Troise, A.D., Mogol, B.A., Roullier, V., Gourdon, A., Jian, S.A.M., Hamzalıoğlu, B.A., Gökmen, V. and Fogliano, V. (2012). Controlling the Maillard reaction by reactant encapsulation: Sodium chloride in cookies, *Journal of Agricultural and Food Chemistry*, 60: 10808-10814.

Gonzalez, P., Tucker, C.J. and Sy, H. (2012). Tree density and species decline in the African Sahel attributable to climate, *Journal of Arid Environments*, 78: 55-64.

Huynh C.T. and Lee D.S. (2014). Controlled release. *In:* S. Kobayashi and K. Müllen (Eds.), *Encyclopedia of Polymeric Nanomaterials*, Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-36199-9_314-1

Islam, M.I.U. and Langrish, T.A.G. (2010). The effect of different atomizing gases and drying media on the crystallization behavior of spray-dried powders, *Drying Technology*, **28**(9): 1035-1043.

Kalušević, A.M., Lević, S.M., Čalija, B.R., Milić, J.R., Pavlović, V.B., Bugarski, B.M. and Nedovic, V.A. (2017a). Effects of different carrier materials on physicochemical properties of microencapsulated grape skin extract, *Journal of Food Science and Technology*, **54**(11): 3411-3420.

Kalušević, A., Lević, S., Čalija, B., Pantić, M., Belović, M., Pavlović, V., Bugarski, B., Milić, J., Žilić S. and Nedović, V. (2017b). Microencapsulation of anthocyanin-rich black soybean coat extract by spray drying using maltodextrin, gum arabic and skimmed milk powder, *Journal of Microencapsulation*, **34**(5): 475-487.

Kayaci, F. and Uyar, T. (2012). Encapsulation of vanillin/cyclodextrin inclusion complex in electrospun polyvinyl alcohol (PVA) nanowebs: Prolonged shelf-life and high temperature stability of vanillin, *Food Chemistry*, 133: 641-649.

Kim, H., Bae, C., Kook, Y.M., Koh, W.G., Lee, K. and Park, M.H. (2019). Mesenchymal stem cell 3D encapsulation technologies for biomimetic microenvironment in tissue regeneration, *Stem Cell Research & Therapy*, 10:51.

Kumari, A., Kaur, B., Srivastava, R. and Sangwan, S.R. (2015). Isolation and immobilization of alkaline protease on mesoporous silica and mesoporous ZSM-5 zeolite materials for improved catalytic properties, *Biochemistry and Biophysics Reports*, 2: 108-114.

Kyriakoudi, A. and Tsimidou, M.Z. (2018). Properties of encapsulated saffron extracts in maltodextrin using the Büchi B-90 nano spray-dryer, *Food Chemistry*, 266: 458-465.

Lalou, S., Mantzouridou, F., Paraskevopoulou, A., Bugarski, B., Levic, S. and Nedovic, V. (2013). Bioflavor production from orange peel hydrolysate using immobilized *Saccharomyces cerevisiae*, *Applied Microbiology and Biotechnolgy*, 97: 9397-9407.

Lević, S., Obradović, N., Pavlović, V., Isailović, B., Kostić, I., Mitrić, M., Bugarski, B. and Nedović, V. (2014). Thermal, morphological, and mechanical properties of ethyl vanillin immobilized in polyvinyl alcohol by electrospinning process, *Journal of Thermal Analysis and Calorimetry*, **118**(2): 661-668.

Lević, S., Lijaković, I.P., Đorđević, V., Rac, V., Rakić, V., Knudsen, T.Š., Pavlović, V, Bugarski, B. and Nedović, V. (2015). Characterization of sodium alginate/D-limonene emulsions and respective calcium alginate/D-limonene beads produced by electrostatic extrusion, *Food Hydrocolloids*, 45: 111-123.

Lević, S., Đorđević, V., Knežević-Jugović, Z., Kalušević, A., Milašinović, N., Branko Bugarski, B. and Nedović, V. (2016). Encapsulation technology of enzymes and applications in food processing, pp. 469-502. *In*: R.C. Ray and C.M. Rosell (Eds.), *Microbial Enzyme Technology in Food Applications*, CRC Press, Taylor & Francis Group, Boca Raton, US.

Lin, S., Lu, Y., Ye, B., Zeng, C., Liu, G., Li, J., Luo, H. and Zhang, R. (2020). Pesticide wastewater treatment using the combination of the microbial electrolysis desalination and chemical-production cell and Fenton process, *Frontiers of Environmental Science & Engineering*, 14: 12.

Mallios, P., Kourkoutas, Y., Iconomopoulou, M., Koutinas, A.A., Psarianos, C., Marchant, R. and Banat, I.M. (2004). Low-temperature wine-making using yeast immobilized on pear pieces, *Journal of the Science of Food and Agriculture*, 84: 1615-1623.

Mao, L., Roos, Y.H., Biliaderis, C.G. and Miao, S. (2017). Food emulsions as delivery systems for flavor compounds: A review, *Critical Reviews in Food Science and Nutrition*, **57**(15).

Marchev, A.S. and Georgiev, M.I. (2020). Plant in vitro systems as a sustainable source of active ingredients for cosmeceutical application, *Molecules*, **25**(9): 2006.

Marchis, T., Cerrato, G., Magnacca, G., Crocella, V. and Laurenti, E. (2012). Immobilization of soybean peroxidase on aminopropyl glass beads: Structural and kinetic studies, *Biochemical Engineering Journal*, 67: 28-34.

Maryšková, M., Schaabová, M., Tománková, H., Novotný, V. and Rysová, M. (2020). Wastewater Treatment by Novel Polyamide/Polyethylenimine Nanofibers with Immobilized Laccase, *Water*, 12: 588.

Matos, M., Gutiérrez, G., Martínez-Rey, L., Iglesias, O. and Pazos, C. (2018). Encapsulation of resveratrol using food-grade concentrated double emulsions: Emulsion characterization and rheological behavior, *Journal of Food Engineering*, 226: 73-81.

McClements, D.J. (2020). Nano-enabled personalized nutrition: Developing multicomponent-bioactive colloidal delivery systems, *Advances in Colloid and Interface Science*, 282: 102211.

Milanovic, J., Manojlovic, V., Levic, S., Rajic, N., Nedovic, V. and Bugarski, B. (2010). Microencapsulation of flavors in carnauba wax, *Sensors*, 10(1): 901-912.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., McClements, D.J., Menard, O., Recio, I., Santos, C.N., Singh, R.P., Vegarud, G.E., Wickham, M.S.J., Weitschies, W. and Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food – An international consensus, *Food & Function*, 5: 1113-1124.

Moejes, S.N., Visser, Q., Bitter, J.H. and van Boxtel, A.J.B. (2018). Closed-loop spray drying solutions for energy efficient powder production, *Innovative Food Science & Emerging Technologies*, 47: 24-37.

Moncada, M., Astete, C., Sabliov, C., Olson, D., Boeneke, C. and Aryana, K.J. (2015). Nano spray-dried sodium chloride and its effects on the microbiological and sensory characteristics of surface-salted cheese crackers, *Journal of Dairy Science*, 98: 5946-5954.

Nedović, V.A., Obradović, B., Leskošek-Čukalović, I., Trifunović, O., Pešić, R. and Bugarski, B. (2001). Electrostatic generation of alginate microbeads loaded with brewing yeast, *Process Biochemistry*, 37: 17-22.

Nedović, V.A., Obradović, B., Poncelet, D., Goosen, M.F.A., Leskošek-Čukalović, I. and Bugarski, B. (2002). Cell immobilization by electrostatic droplet generation, *Landbauforschung Volkenrode* SH, 241: 11-17.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S. and Bugarski, B. (2011). An overview of encapsulation technologies for food applications, 11th International Congress on Engineering and Food (ICEF11), Athens, Greece, *Procedia Food Science*, 1: 1806-1815.

Patterson, D.P., Schwarz, B., Waters, R.S., Gedeon, T. and Douglas, T. (2014). Encapsulation of an enzyme cascade within the bacteriophage P22 virus-like particle, *ACS Chemical Biology*, **9**(2): 359-365.

Prüsse, U., Bilancetti, L., Bučko, M., Bugarski, B., Bukowski, J., Gemeiner, P., Lewinska, D., Manojlovic, V., Massart, B., Nastruzzi, C., Nedovic, V., Poncelet, D., Siebenhaar, S., Tobler, L., Tosi, A., Vikartovská, A. and Vorlop, K.D. (2008). Comparison of different technologies for alginate beads production, *Chemical Papers*, 62: 364-374.

Puig-Pujol, A., Bertran, E., García-Martínez, T., Capdevila, F., Mínguez, S. and Mauricio, J.C. (2013). Application of a new organic yeast immobilization method for sparkling wine production, *American Journal of Enology and Viticulture*, 64: 386-394.

Radosavljević, M., Lević, S., Belović, M., Pejin, J., Djukić-Vuković, A., Mojović, Lj. and Nedović, V. (2020). Immobilization of *Lactobacillus rhamnosus* in polyvinyl alcohol/calcium alginate matrix for production of lactic acid, *Bioprocess and Biosystems Engineering*, 43: 315-322.

Reddy, L., Reddy, Y., Reddy, L. and Reddy, O. (2008). Wine production by novel yeast biocatalyst prepared by immobilization on watermelon (*Citrullus vulgaris*) rind pieces and characterization of volatile compounds, *Process Biochemistry*, 43: 748-752.

Reddy, L.V., Reddy, L.P., Wee, Y.J. and Reddy, O.V.S. (2011). Production and characterization of wine with sugarcane piece immobilized yeast biocatalyst, *Food Bioprocess Technology*, 4: 142-148.

Salević, A., Prieto, C., Cabedo, L., Nedović, V. and Lagaron, J.M. (2019). Physicochemical, antioxidant and antimicrobial properties of electrospun poly(ε-caprolactone) films containing a solid dispersion of sage (*Salvia officinalis* L.) extract, *Nanomaterials*, 9: 270.

Santana, R.C., Perrechil, F.A. and Cunha, R. L. (2013). High- and low-energy emulsifications for food applications: A focus on process parameters, *Food Engineering Reviews*, 5: 107-122.

Šeregelj, V., Tumbas-Šaponjac, V., Mandić, A., Ćetković, G., Čanadanović-Brunet, J., Vulić, J. and Stajčić, S. (2018). Accelerated solvent extraction of bioactive compounds from carrot – Optimization of response surface methodology, *Journal of Serbian Chemical Society*, **83**(11): 1223-1228.

Sobel, R., Versic, R. and Gaonkar, A.G. (2014). Introduction to microencapsulation and controlled delivery in foods, pp. 3-12. *In*: A. Gaonkar, N. Vasisht, A. Khare and R. Sobel (Eds.), *Microencapsulation in the Food Industry: A Practical Implementation Guide*, Academic Press (Elsevier Inc.), San Diego, USA.

Stajić, S., Stanišić, N., Lević, S., Tomović, V., Lilić, S., Vranić, D., Jokanović, M. and Živković, D. (2018). Physico-chemical characteristics and sensory quality of dry fermented sausages with flaxseed oil preparations, *Polish Journal of Food and Nutrition Sciences*, 68: 367-375.

Stajić, S., Živković, D., Tomović, V., Nedović, V., Perunović, M., Kovjanić, N., Lević, S. and Stanišić, N. (2014). The utilization of grapeseed oil in improving the quality of dry fermented sausages, *International Journal of Food Science & Technology*, **49**(11): 2356-2363.

Su, J., Cai, Y., Tai, K., Guo, Q., Zhu, S., Mao, L., Gao, Y., Yuan, F. and Van der Meeren, P. (2021). High-internal-phase emulsions (HIPEs) for co-encapsulation of probiotics and curcumin: Enhanced survivability and controlled release, *Food & Function* (in press).

Su, Y., Sun, C., Sun, X., Wu, R., Zhang, X. and Tu, Y. (2020). Acetylresveratrol as a potential substitute for resveratrol dragged the toxic aldehyde to inhibit the mutation of mitochondrial DNA, *Applied Biochemistry and Biotechnology*, 191: 1340-1352.

Thies, C. (2005): *Microencapsulation. Kirk-Othmer Encyclopedia of Chemical Technology*, 4th edition, 16: 317-327.

Ton, N.M.N. and Le, V.V.M. (2011). Application of immobilized yeast in bacterial cellulose to the repeated batch fermentation in wine-making, *International Food Research Journal*, 18: 983-987.

Tucker, N., Stanger, J.J., Staiger, M.P., Razzaq, H. and Hofman, K. (2012). The History of the Science and Technology of Electrospinning from 1600 to 1995, *Journal of Engineered Fibers and Fabrics*, special edition, 7: 63-73.

Vafania, B., Fathi, M. and Soleimanian-Zad, S. (2019). Nanoencapsulation of thyme essential oil inchitosan-gelatin nanofibers by nozzle-less electrospinning and their application to reduce nitrite in sausages, *Food and Bioproducts Processing*, 116: 240-248.

Vázquez-León, L.A., Olguín-Rojas, J.A., Páramo-Calderón, D.E., Gerardo, F. Barbero, Salgado-Cervantes, M.A., Miguel Palma, García-Alvarado, M.A. and Rodríguez-Jimenes, G.C. (2020). Closed-loop spray drying with N_2 of *Moringa oleifera* leaf ethanolic extracts: Effects on bioactive compounds and antiradical activity, *Drying Technology*, DOI: 10.1080/07373937.2020.1753764

Verbelen, P.J., Nedović, V.A., Manojlović, V., Delvaux, F.R., Laskošek-Čukalović, I., Bugarski, B. and Willaert, R. (2010). Bioprocess intensification of beer fermentation using immobilized cells, pp. 303-326. *In*: N.J. Zuidam and V.A. Nedovic (Eds.), *Encapsulation Technologies for Food Active Ingredients and Food Processing*, Springer, Dordrecht.

Veršič, J.R. (2014). The economics of microencapsulation in the food industry, pp. 409-420. *In*: A. Gaonkar, N. Vasisht, A. Khare and Sobel R. (Eds.), *Microencapsulation in the Food Industry: A Practical Implementation Guide*, Academic Press (Elsevier Inc.), San Diego, USA.

Vulić, J., Šeregelj, V., Kalušević, A., Lević, S., Nedović, V., Tumbas-Šaponjac, V., Čanadanović-Brunet, J. and Ćetković, G. (2019). Bioavailability and bioactivity of encapsulated phenolics and carotenoids isolated from red pepper waste, *Molecules*, 24: 2837.

Wandrey, C., Bartkowiak, A. and Harding, E.S. (2010). Materials for Encapsulation, pp.

31-100. *In*: N.J. Zuidam and V.A Nedovic (Eds.), *Encapsulation Technologies for Food Active Ingredients and Food Processing*, Springer, Dordrecht.

Xu, L., Weathers, P.J., Xiong, X.R. and Liu, C.Z. (2009). Microalgal bioreactors: Challenges and opportunities, *Engineering* in *Life Sciences,* **9**(3): 178-189.

Yilmaztekin, M., Lević, S., Kalušević, A., Cam, M., Bugarski, B., Rakić, V., Pavlović, V. and Nedović, V. (2019). Characterization of peppermint (*Mentha piperita* L.) essential oil encapsulates, *Journal of Microencapsulation*, **36**(2): 109-119.

Zhu, F. (2017). Encapsulation and delivery of food ingredients using starch based systems, *Food Chemistry,* 229: 542-552.

Zimmermann, M.B. and Windhab, E.J. (2010). Encapsulation of iron and other micronutrients for food fortification, pp. 187-210. *In*: N.J. Zuidam and V.A. Nedovic (Eds.), *Encapsulation Technologies for Food Active Ingredients and Food Processing*, Springer, Dordrecht.

Zuidam, N.J. and Shimoni, E. (2010). Overview of microencapsulates for use in food products or processes and methods to make them, pp. 3-29. *In*: N.J. Zuidam and V.A. Nedovic (Eds.), *Encapsulation Technologies for Food Active Ingredients and Food Processing*, Springer, Dordrecht.

CHAPTER

2

Carrier Materials for Encapsulation

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1. Introduction

Encapsulation may be defined as ‘a technology for packaging small solid particles, liquid droplets, or gas molecules in a form that can release the contents at controlled rates under specific conditions and/or upon receiving a certain stimulus’ (Desai and Park, 2005; Picot and Lacroix, 2003). Encapsulation technology is evolving and many materials are encapsulated to protect them from various harmful factors, as well as to deliver specific materials to the targeted areas without getting effected through their journey. Among different encapsulation techniques, microencapsulation is used widely in food industry, where solids, liquid or gaseous material are packed into microcapsules, using polymers as coating material (Gharsallaoui *et al*., 2007; Wandrey *et al*., 2010).

In the ideal case, the shell material should have the following properties:

1. It should have good rheological properties and easy to handle during encapsulation process.
2. It should be soluble in solvents acceptable in the food industry, e.g. water, ethanol, etc.
3. It should have the capability to hold the active material in stable emulsion form during processing and storage.
4. It should not react with active material during processing or storage.
5. It should completely release the active material.
6. It should provide maximum protection to the active material against environmental conditions (e.g. heat, light, humidity).
7. It should be economically sustainable and food-grade substance (Shahidi and Han, 1993).

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Ever since Barrett Green developed the encapsulation technique using gelatin as carrier material (Fanger, 1974), many carrier materials have been tried and tested. A plethora of substances (of different types, origins and properties), including natural or synthetic, are available and could be employed as microcapsule shell material, but most of the commercially prepared microcapsules employ a rather small number of shell materials. The reason for this is that only materials certified as 'generally recognized as safe' (i.e. under the GRAS standard) should be used in the food encapsulation processes. The precise safety requirements for the quality of shell materials are defined by the agencies, such as the European Food Safety Authority (EFSA) or Food and Drug Administration (FDA) in the USA (Wandrey *et al*., 2010; Gibson *et al*., 2017).

The majority of materials used for microencapsulation in the food sector are bio-based materials, such as carbohydrates, proteins, lipids and miscellaneous materials (Mishra, 2015; Chaudhary and Patel, 2019). A brief introduction of various materials that are used as carrier materials is given below.

1.1 Polysaccharides

The most widely used encapsulating materials in the food industry belong to the group of polysaccharides. These polysaccharides can be divided into plant-based and animal-based materials, as given in Table 1. Their wide usage in food industry is due the fact that they have desirable physicochemical properties, such as solubility, melting, phase change and can be used to form different shapes in a wide range of sizes. Another important aspect in their selection is that they are very cost-effective materials (Brownlie, 2007).

1.2 Proteins

Proteins are natural biomolecules made up of linear chains of amino acids and are used in encapsulation and other applications in the food industry. There are several types of proteins available. Application of proteins has some advantages, such as biocompatibility, biodegradability, good water solubility, emulsifying properties and foaming capacity. Application of vegetable proteins as shell material in microencapsulation is a trend in the pharmaceutical, cosmetics and food industries. This is because vegetable proteins are identified to be less allergenic when compared to animal proteins (Modi and Seth, 2010). Proteins can encapsulate hydrophobic and hydrophilic compounds alone, but also can be mixed with polysaccharides or synthetic polymers. Disadvantage of proteins as encapsulating agents is due to their low solubility in cold water, probability to react with carbonyls and above all, an important factor is cost, which is higher when compared with polysaccharides and this limits proteins application in food encapsulation processes (Shahidi and Han, 1993).

Proteins which are employed as shell materials can also be divided into two groups, based on their origin (Table 2).

1.3 Lipids

Lipids, such as fats, fatty acids, waxes, and phospholipids are used as edible coating materials. Among the lipids, those that exhibit amphiphilic properties, i.e. the molecules which reduce the surface tension of the medium or reduce interfacial tension between phases at which they are adsorbed, are very useful for encapsulation of active compounds. Due to low polarity of lipids, they obstruct the moisture

Table 1: Polysaccharides Used as Shell Materials for Encapsulation

Plant Origin	Animal and Microbial Origin	Marine Origin
Starch derivatives: Amylose, Amylopectin,	Xanthan	Alginate
Dextrins, Maltodextrins, Polydextrose	Gellan	Carrageenan
Octenyl succinic anhydride modified starch	Dextran	
Sodium starch octenyl succinate,	Chitosan	
Pearl millet starch	Curdlan	
Arrowroot starch	Pullulan	
Sorghum starch		
Cellulose derivatives: Methylcellulose,		
Hydroxypropyl cellulose		
Hydroxypropyl methylcellulose		
Carboxymethyl cellulose		
Plant exudates: Gum arabic		
Gum Tragacanth		
Gum Karaya		
Mesquite Gum		
Gum Angum		
Plant extracts: Galactomannans		
Pectins		
Soluble soybean polysaccharides		
Mucilage		
Prebiotics polysaccharides: Inulin		
Fructooligosaccharides (FOS)		
Galactooligosaccharides (GOS)		
Xylooligosaccharides (XOS)		

Table 2: Plant- and Animal-based Proteins Used as Shell Materials for Encapsulation

	Plant Based	Animal Based
Cereal	Oat protein	Casein and caseinates
	Gluten and gliadins – wheat	Whey proteins
	Barley protein	Gelatin
	Zein (corn)	Collagen
	Rice bran	Elastin
Soy and Pulses	Soy protein	Albumin
	Pea protein	
	Chickpea protein	
	Lentil protein	
Miscellaneous	Sunflower protein	
	Gliadin	
	Lectin	
	(Martins *et al.*, 2018)	

transport. However, their hydrophobic nature confers fragility to the formed coatings. Thus, lipids should be melded/mixed with proteins or polysaccharides, in order to improve their coating characteristics. Some of the lipid materials that are utilized in food encapsulation are partial acylglycerols, phospholipids, glycolipids, aminolipids and lipopeptides, phytosterol surfactant, and antioxidant esters, tristearic acid, diglycerides, monoglycerides, oils, fats, hardened oils, wax, paraffin, natural waxes, such as beeswax, paraffin waxes and oxidized polyethylene waxes (synthetic waxes).

1.4 Synthetic Polymers

Natural polymers have certain disadvantages, such as rigid structure that limits potential chemical modifications; also, during extraction of natural polymers, unwanted remnants (e.g. impurities) may remain. The quality of the extracted natural polymers may also vary from batch to batch. To overcome these disadvantages, synthetic polymers, like PVA, PEG, polycaprolactone, PLGA, isocyanates, polyamide, polyurea, polyurethane, melamine formaldehyde, poly (vinyl pyrrolidone) and poly (vinyl acetate-co-crotonic acid) are usually employed for encapsulation of active compounds. They possess greater mechanical and chemical stability, increased reproducibility due to the minimized batch-to-batch variation, reduced non-specific protein binding and could be easily modified, providing carrier materials and encapsulates with tunable properties (Lu *et al.*, 2007; Young *et al.*, 2012).

There are numerous books and copious publications dealing comprehensively with different biopolymers, polysaccharides, hydrocolloids, or gums, and their use in encapsulation process. The aim of the current chapter is to present and update the existing knowledge about different encapsulating materials either already in use or with the potential for application in food sector. Considering that other authors of this book will cover various aspects of encapsulation in the food sector, in this chapter, we primarily focus on the shell materials reviewing and current state-of-the art in the area of probiotics encapsulation, especially focusing on prebiotics as carrier materials for encapsulation.

2. Prebiotics

Prebiotics are materials that are actually used to enhance the growth of probiotics; in other words, prebiotics serve as probiotics' food. Prebiotics are defined as 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon the host's well-being and health' (Roberfroid, 2007). Much accepted definition of prebiotics is given by the International Scientific Association for Probiotics and Prebiotics (ISAPP), where they are defined as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit'. The term 'prebiotic' may also encompass non-carbohydrates' varied classes other than foods which can act on the entire body of both humans and animals by managing the microorganisms for maintaining health and prevent diseases (Gibson *et al.*, 2017). These prebiotic compounds are usually relatively short chained, possess low molecular-weight carbohydrates that are non-active food constituents and are selectively fermented in the colon, especially by bifidobacteria and lactic acid bacteria. These bacteria utilize the prebiotic compounds, providing essential nutrients and energy (Gourineni *et al.*, 2011; Salvini *et al.*, 2011). They improve the host's health by improving the survival, growth, metabolism and beneficial health activities of probiotics in the digestive system. They are antagonistic to pathogenic organisms, limiting their proliferation (Yee *et al.*, 2019). Regarding the effects of prebiotics on probiotic microorganisms, the logical approach in the development of new food formulations would be to combine these two beneficial nutritional components via encapsulation. In literature, a term *synbiotic encapsulation* (combination of prebiotic carrier(s) and probiotic cells) is used to define an efficient system for targeted delivery of probiotics (Wu and Zhang, 2018).

Addition of prebiotic components (e.g. 'Raftilose P95'and polydextrose) was reported to offer protection to probiotics with improved stability (Capela *et al.*, 2006; Riaz and Masud, 2013; Martinez *et al.*, 2015). Specific prebiotic compound has the ability to augment a particular group of probiotic bacteria in certain intestinal regions (Okuro *et al.*, 2013; Sathyabama *et al.*, 2014). Using this beneficial aspect, utilization of prebiotic for micro capsule preparation could help the probiotic organism to survive under unfavorable conditions; also, encapsulation into prebiotics may help probiotics to grow better in the GI tract where they get additional advantage to compete and grow in the complex environment (Okuro *et al.*, 2013; Sathyabama *et al.*, 2014). Various prebiotic compounds, like inulin, FOS, GOS, inulooligosaccharides (IOS) and XOS have been used in preparation of microcapsules, which could be further applied for preparation of fermented dairy products, like yogurt, without reducing the required probiotic count. Various prebiotics used as carrier material for probiotics encapsulation are diagrammatically represented in Fig. 1. These encapsulated probiotics could also survive simulated gastrointestinal conditions (Li *et al.*, 2020). Additionally, co-encapsulation of probiotic isolate can be a new trend, which might reduce the cost of using prebiotics (Chen *et al.*, 2015).

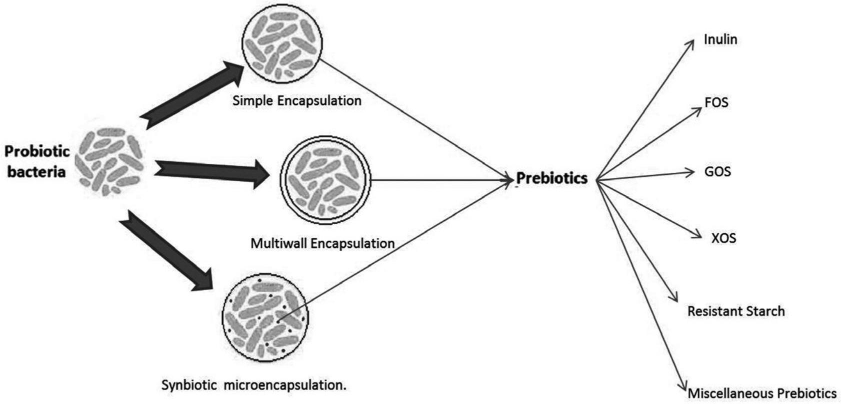

Fig. 1: Prebiotics used as carrier material for probiotics encapsulation

There are some reports which suggest that application of prebiotics as encapsulating material or as co-encapsulant can improve the probiotics' survivability in extreme conditions prevailing in acidic products and frozen products as shown in Table 3.

2.1 Inulin

Inulins are a group of naturally occurring non-structural, storage carbohydrates formed by many types of plants (Abed *et al.*, 2016). The term inulin refers to a heterogeneous blend of fructose polymers, comprising of β-d-fructosyl which residues with (2→1) linkages and generally has a (1→2) linked α-d-glucosyl terminal residue. Inulins are present in commonly consumed fruits and vegetables, like onion, leek, banana, wheat, garlic and rye (Vijn and Smeekens, 1999; Niness 1999; Roberfroid, 2005; Kumar *et al.*, 2015). Survival of probiotics in harsh acidic environments was enhanced in the presence of inulin (Yee *et al.*, 2019). Microcapsules containing inulin have the ability

Table 3: Studies Dealing with the Microencapsulation of Probiotics with Prebiotics as Coating Material Applied in Food Products

Product	Prebiotic and/or Probiotic Types	Major Outcome(s)	Reference
Yogurt	Galacto-oligosaccharide (GOS) and lactitol (LAC)	Microencapsulation and addition of LAC help to enhance the survival of probiotic strains in yogurt during storage, but had a negative relationship between the addition of synbiotic microcapsules and the improvement of yogurt quality.	Li *et al.*, 2020
	Carrageenan	Encapsulation improved the probiotics viability in the prepared yogurt and gastrointestinal tract. In the case of encapsulated bacteria, only 3 logs while for free cells, 7 log reductions were recorded. However, sodium alginate microcapsules exhibited better release profile than carrageenan.	Afzaal *et al.*, 2019
	Inulin in free form or in the form of nanoparticles encapsulated bifidobacterium cultures	The viability of *Bifidobacterium* cultures was enhanced when double-coated microcapsule was applied compared to the free one. Moreover, the enhancement was boosted when inulin was incorporated in either free form or in the form of nanoparticles.	Fayed *et al.*, 2019
Synbiotic diet mousse	Inulin *Lactobacillus acidophilus* La-5	After 6 h of the *in vitro* assays, the lowest reduction of cell counts occurred for mousse with microencapsulated cells (1.3 log cycles), followed by 30 microencapsulated cells (2.0 log cycles), mousse with free cells (3.0 log cycles), and free cells (7.4 log cycles).	dos Santos *et al.*, 2019
Symbiotic chewing gum	Alginate, inulin (0–1%) and lecithin (0–1%). *Lactobacillus reuteri*	The viability of the probiotic in encapsulated samples was retained after 21 days unlike control.	Qaziyani *et al.*, 2019
Functional blueberries	Alginate-based coatings enriched with inulin and oligofructose *Lactobacillus rhamnosus* CECT 8361	All the prebiotics showed enhancement of probiotic viability with counts above 6.2 log CFU/g for the entire period, whereas counts of native microbiota remained under safe levels. Overall visual quality, odor and flavor were acceptable up to storage of 14 days.	Bambace *et al.*, 2019

of self-aggregation and form an insoluble mass inside Ca-alginate matrix, delaying the penetration of H+ ions into the microcapsules by blocking the pores (Zaeim *et al.*, 2019). Atia *et al.* (2017, 2018) reported that co-encapsulation of *lactobacilli* with 50mg/g inulin maintained viability of bacteria in acidic conditions. Encapsulation with inulin increased the encapsulation efficiency of probiotic *Lactobacillus acidophilus*. Microcapsule showed good resistance to simulated gastrointestinal conditions and viability of probiotics was found to be stable even at −18°C for 120 days (Poletto *et al.*, 2019). Another study carried out by Krasaekoopt and Watcharapoka (2014) showed improved viability of the probiotic *Lb. acidophilus* under simulated GI conditions when co-encapsulated with inulin and chitosan coated with Ca-alginate.

When *Lb. casei* was mixed with inulin and then encapsulated with 2 per cent sodium alginate, 98 per cent encapsulation efficiency was observed, while the synbiotic microcapsules' size was around 24µm. Encapsulated probiotic showed good resistance to gastrointestinal liquids *in vitro* (Banerjee *et al.*, 2017). In another study, *Lactobacillus rhamnosus* GG was encapsulated with inulin, alginate and chitosan; encapsulation contributed to a significant improvement in the survival of the probiotic organism under simulated gastrointestinal conditions. Such encapsulates when added to apple juice, improved its sensory properties during 90 days of storage at 4 and 25°C (Gandomi *et al.*, 2016).

Lb. plantarum and *Bifidobacterium lactis*, when co-encapsulated with inulin in calcium alginate and chitosan, exhibited encapsulation efficiency up to 78.9 per cent. Inulin is found to leech out of the matrix, but it also showed that chitosan reduced the release of inulin from the matrix. The presence of inulin ensured that cells have good resistance toward harsh gastrointestinal conditions as compared to free probiotic organisms. However, the efficiency of encapsulation process also depends on the characteristics of probiotic strains as well as on storage conditions. Under the same conditions, *B. lactis* was found to be highly sensitive to environment and even co-encapsulation with inulin did not improve *B. lactis* survival during storage at 25°C, but survivability was enhanced when this encapsulated strain was stored at 4°C and −18°C for 90 days. *Lb. plantarum* showed little loss of viability when stored at 4°C and −18°C, and satisfactory viability after storage at 25°C for 90 days (Zaeim *et al.*, 2019).

Encapsulated *Lactobacillus plantarum* with inulin-sodium alginate coated with skim milk showed good resistance towards freeze-drying and greater resistance to simulated gastric fluid. Loss of viability was also found to be less after 7 weeks of storage period. Gene expression related to probiotic functionality was also found to be unaffected when inulin was incorporated as an encapsulant (Wang *et al.*, 2016).

Microcapsules prepared with sweet whey and inulin provided good protection to *Bifidobacterium* BB-12 (Pinto *et al.*, 2015). *Bifidobacterium* BB-12 was also found to survive in simulated gastrointestinal conditions and was able to show antagonistic activity towards *E.coli* when inulin and goat milk powder were used as carrier materials (Verruck *et al.*, 2020). *Saccharomyces cerevisiae* var. *boulardii* encapsulated in inulin, xanthan gum and alginate survived and grew during 4 weeks of storage at 4°C in berry juice (Fratianni *et al.*, 2014).

Researchers established that the survivability of the encapsulated *Bifidobacterium* in the simulated gastric solution could be significantly higher than the survivability of the free bacteria when alginate and arabic gum are used in combination with inulin nanoparticles as carriers (Fayed *et al.*, 2018). Further, other investigations also confirmed the significance of applying multi-particulate microcapsule for